Effect of Cold Treatment on Survival and Development of Codling Moth (Lepidoptera: Tortricidae) in Cherry

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ABSTRACT 'Bing' cherries, *Prunus avium* L., were obtained from an organic orchard and a conventional commercial orchard. The two groups were examined separately in replicated tests infested with each fruit initially infested with a first-instar codling moth, *Cydia pomonella* (L.). To simulate commercial postharvest holding conditions, the treatments were 0 (control), 1, 2, 4, 7, 10, and 14 d cold storage at 3.3°C. The fruits were examined three or more times to determine larval survival, life stage, fruit condition, and fungal disease. Survival of first instars was affected only by cold storage durations of ≥7 d. When infested with codling moth larvae, both organic and conventionally grown cherries quickly deteriorated from fungal diseases. The rate of moth development was estimated from the surviving larvae and was significantly different between organic and conventionally grown cherries for all instars except the second.

KEY WORDS *Cydia pomonella*, postharvest biology, commodity, life history, quarantine.

JAPANESE QUARANTINE REGULATIONS against the codling moth, *Cydia pomonella* (L.), require that sweet cherries, *Prunus avium* L., from the United States be fumigated with methyl bromide before export to Japan (Sell et al. 1988, Moffitt et al. 1992). Because cherries are perishable, they are held in cold storage (up to 2 wk) until marketing and are generally air-freighted to Japan. Regulations also prevent the application of fungicides to the fruits. Although intensive inspections have been proposed to assure pest-free fruits, Japanese regulators are concerned that eggs laid in the stem area may be overlooked by inspectors. Larval feeding, however, is detectable. Observations made in an earlier study on infested cherries (Hansen et al. 2000) indicated that codling moth larvae older than second instar form obvious tunnels. Wearing (1979) also separated instars by their characteristic tunneling.

Documentation of the codling moth-cherry relationship is rare. Mote (1926) reported finding "codling moth-like larvae" in cherries from western Oregon. In caged studies, Hagley et al. (1980) found almost all codling moth eggs on foliage, rather than fruits, and reported that codling moths had not been recovered from sour cherry, *Prunus cerasus* L., for the previous 8 yr. In quarantine studies, codling moths were forced to infest cherries by either releasing adult moths in caged trees with fruit (Anthon et al. 1975) or in the laboratory with containers of fruit (Maindonald et al. 1992), or by individually placing larvae by hand on each fruit (Hansen et al. 2000). However, in none of these studies were the insects allowed to develop to maturity. Other published observations on codling moth infesting cherries are scarce and there is no established method for rearing codling moth in cherries. If cherries are a true host of codling moth, then the insects should be easily reared from the fruits, as can be done with apples.

In conventional commercial cherry orchards of the Pacific Northwest, insecticides are applied to control cherry fruit flies, *Rhagoletis* spp. (Diptera: Tephritidae), rather than codling moth (Roberts 1998). Furthermore, cherries intended for export are regularly inspected by the Washington State Department of Agriculture for cherry fruit flies and codling moth. From 1983 to 1996, no codling moth has been reported on any of the examined cherries (7.57×10^7 cherries) (Wearing et al. 2001). A few cherries are organically grown, but these are not exported.

Although fresh sweet cherries are nationally worth U.S. $15 million, there is an apparent dearth of information on how this fruit is related to one of its primary quarantine pests, the codling moth. Considerable time and resources are expended to control the codling moth in cherry, yet its complete life cycle has never been documented in that fruit. Thus, it would be beneficial to obtain basic information on the interactions between cherries and codling moth.

Thus, the three objectives of this study were as follows: (1) to examine the development and survival of codling moth in cherry; (2) to compare codling moth development in organic and conventionally pro-

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duced cherries; (3) to determine if short-time cold storage reduces survival of early instars of the codling moth.

Materials and Methods

Fruit Sources. Mature 'Bing' cherries, representing fruits from two different growing regimes from central Washington, were obtained locally from commercial sources. One group was organically grown. The other group was grown under conventional pest management systems, which included regular insecticide applications for cherry fruit flies, *Rhagoletis* spp. (Roberts 1998). Samples from both groups were analyzed by the Laboratory Services of Washington State Department of Agriculture (Yakima, WA) for synthetic insecticidal residues. They found that the organically grown cherries had no detectable residues whereas the conventional cherries contained 0.35 ppm of carbaryl and traces of azinphos-methyl.

Treatment Procedures. Codling moth newly hatched larvae were obtained from a colony reared at the USDA-ARS Yakima Agricultural Research Laboratory and maintained on a soya-wheat germ-starch artificial diet (Toba and Howell 1991) at ≈27°C, 40–50% RH, with a photoperiod of 16:8 (L:D) h. We placed a neonate larva near the stem end of each cherry. We placed each infested cherry alone in a tapered plastic cup (3 cm bottom diameter, 4 cm top diameter, 4 cm high) with a plastic top. Each treatment used 50 cherries with each in an individual cup. After consultation with industry representatives, treatment temperature was selected at 3.3°C and treatment durations were for 0 (control), 1, 2, 4, 7, 10, and 14 d. The treatments were replicated four times for both groups of cherries. Thus, for every organic and conventionally grown cherry, there were seven treatments, each with four replicates of 50 fruits. After treatment, the infested cherries were maintained in a 25°C rearing room.

Data Collection. The infested cherries were observed at least three times at different intervals. Larval viability, instar, condition of the fruit, and presence of fungal disease were recorded for each observation. Excess fluids were drained off, as necessary, as the cherries deteriorated due to fungal disease. If a cherry was destroyed by fungi, the larva was extracted (if found) and placed alone on an immature organically grown apple in a separate container, a tapered plastic cup described above. This is routine for enhancing larval survival in laboratory studies and is required by Japan for treatment tests against codling moth (Hansen et al. 2000). Missing larvae were assumed dead as is standard procedure when evaluating quarantine treatments against the codling moth (Moffitt et al. 1992, Hansen et al. 2000). Corrugated cardboard squares were attached to the inner side of the lid tops to provide pupation sites for larvae in apples and cherries. Cocooned fifth instars were noted, but larval development was considered complete when the insects pupated. After pupation, fruits were removed from the containers and cardboard squares held for moth emergence.

Table 1. Numbers (mean ± SE) of organically grown and conventionally produced cherries artificially infested with codling moth larvae and showing fungal disease after different durations of cold storage

Cold storage (days)	Organic No. diseased	Conventional No. diseased	*t*-value
0	48.5 ± 0.5	39.0 ± 1.7	24.00**
1	49.3 ± 0.3	39.0 ± 3.1	24.00**
2	48.0 ± 0.7	41.8 ± 2.5	13.11*
4	46.8 ± 0.5	35.8 ± 4.4	20.21**
7	44.0 ± 2.3	32.3 ± 1.1	19.20**
10	37.8 ± 1.9	25.8 ± 1.2	20.21**
14	32.5 ± 3.3	17.3 ± 1.8	19.20**

Results from Wilcoxon rank sum tests between means from organic and conventional cherries are indicated by *, significant difference at $P < 0.05$ and **, significant difference at $P < 0.01$; means are from four replicates with $n = 50$ cherries/replicate.

Data Analysis. Data were analyzed using SAS (SAS Institute 1989). To estimate total developmental time based on instar, the maximum accumulated days from infestation were determined for every instar for each insect by using the MAX function in PROC MEANS. This provided the total duration for each and all proceeding instars. Because some replicates had few or no survivors, the level of survivorship was calculated by pooling the data among the replicated for each cold storage regime and expressing the proportion (the number of insects completing an instar divided by the number of insects entering that instar) as a percentage. Stadia were estimated by the amount of time between successive instars. Life stages of missing larvae were estimated by comparing the last observation with the duration of accumulated stadia for each instar.

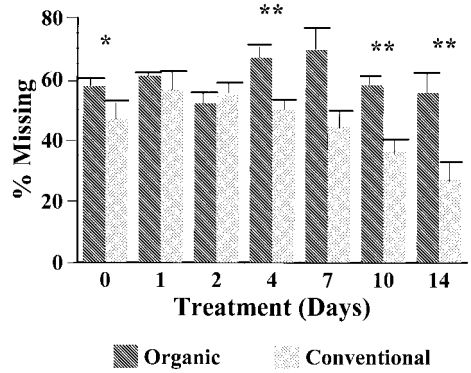

Fig. 1. Percentage (mean ± SE) of larvae missing during observations on larval development in organically grown and conventionally produced cherries after different durations of cold storage at 3.3°C. Results from Wilcoxon rank sum tests between means from organic and conventional cherries are indicated by * equal significant difference at $P < 0.05$ and ** equal significant difference at $P < 0.01$; means are from four replicates with $n = 50$ cherries/replicate.

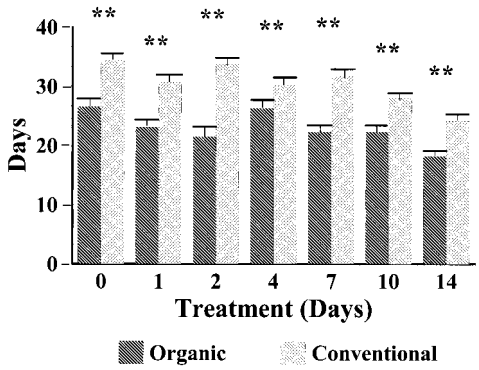

Fig. 2. Number of days (mean ± SE) to first observation of missing codling moth larvae from organically grown and conventionally produced cherries reared at 25°C after different durations of cold storage at 3.3°C. Results from Wilcoxon rank sum tests between means from organic and conventional cherries are indicated by ** equal significant difference at $P < 0.01$; means are from four replicates with $n = 50$ cherries/replicate.

Univariate statistics were calculated by using PROC MEANS (SAS Institute 1989). Paired *t*-test comparisons were performed by obtaining the differences between paired variables and using the T and PRT options of PROC MEANS. Nonparametric tests were used to determine significant differences among variables by first arranging data by PROC RANK, then performing PROC GLM. This is the equivalent to a Wilcoxon rank sum test for two samples and the Kruskal-Wallis *k*-sample test for more than two samples (Zolman 1993).

Results and Discussion

Diseased Fruits. Cherries used in this study were not treated with fungicide to simulate the types of fruits exported to Japan. However, both organic and conventionally grown cherries were highly prone to fungal infections. Although organic cherries were significantly more susceptible to fungal disease than the conventionally produced cherries for each cold storage treatment, the level of infection decreased with cold storage for both organic and conventionally grown fruits (Table 1). However, the factors involved with fungal infections are complex and there was insufficient information from this study to determine the most important species, but the fungi formed obvious multiple colonies and probably consisted of a complex of species including *Alternaria*, *Botrytis*, *Cladosporum*, *Penicillium*, and *Rhizopus* spp. (Harvey et al. 1972). The infected cherries were covered quickly with thick fungal layers over all solid components while the cups filled with fluid from the deteriorated fruits. Under such conditions, live larvae were difficult to locate and larval cadavers were quickly destroyed. Microscopic examinations of larvae from diseased fruits revealed abnormal spots on the larval cuticle, perhaps characteristic of unsuccessful fungal entries. Live larvae were seen on the least damaged structures of the fruits, usually the stems. These larvae were transferred to immature apples. In a commercial setting, cherries exported to Japan would not be treated with fungicides. Diseased fruits would be eliminated at the packing line or rejected at least by the time that the fruits reach the retail level. Furthermore, diseased fruits would occur in clumps within sealed plastic shipping bags, which would result in all the contents becoming infected.

Table 2. Comparison of accumulated developmental time in days (mean ± SE) estimated for each instar of the codling moth reared from organically grown and conventionally produced cherries

From infestation through instar	Organic		Conventional		*t*-value
	No. of days	No. larvae	No. of days	No. larvae	
1	4.4 ± 0.4	213	5.1 ± 0.2	371	17.04**
2	10.2 ± 1.0	47	10.0 ± 1.7	34	2.79
3	17.9 ± 1.0	88	23.3 ± 1.3	28	11.52**
4	22.7 ± 0.8	135	28.0 ± 0.7	118	17.95**
5	32.0 ± 1.9	63	36.6 ± 1.0	179	5.81*

Data were pooled from all cold storage regimes. Results from Wilcoxon rank sum tests between means from organic and conventional cherries are indicated by *, significant difference at $P < 0.05$ and **, significant difference at $P < 0.01$; means are from pooled total number of larvae.

The percentage of missing larvae was > 50% for all treatment durations for the organic cherries (Fig. 1) and the numbers of missing larvae were not significantly different among the storage treatments ($F = 2.14$; df = 6, 21; $P > 0.05$). The percentage of missing larvae significantly decreased with increased cold storage durations among the conventional cherries ($F = 4.86$; df = 6, 21; $P < 0.05$), with the lowest percentage of missing larvae (27%) in cherries previously stored for 14 d.

The loss of larvae was discovered sooner in organic cherries than in conventional cherries (Fig. 2). The time after treatment that larvae were first noticed to be missing among the storage treatments within a cherry type was significantly different for organic cherries ($F = 4.80$; df = 6, 833; $P < 0.01$) and conventional cherries ($F = 6.70$; df = 6, 626; $P < 0.01$). The number of days after treatment that larvae were first

Table 3. Numbers (mean ± SE) of codling moths completing larval development in organically grown and conventionally produced cherries after different durations of cold storage at 3.3°C

Cold storage, d	No. of larvae			
	Organic		Conventional	
0	7.0bc	1.1	15.5a	2.7
1	9.8ab	1.9	12.5ab	3.1
2	15.8a	2.6	15.0ab	2.9
4	9.8ab	2.7	13.8ab	2.4
7	5.2bc	2.1	12.0ab	2.9
10	4.0cd	1.3	8.0bc	1.3
14	0.8d	0.2	5.0c	0.9

Means within columns followed by the same letter are not significantly different by LSD separation ($P < 0.05$). Means represent four replicates each with $n = 50$ larvae.

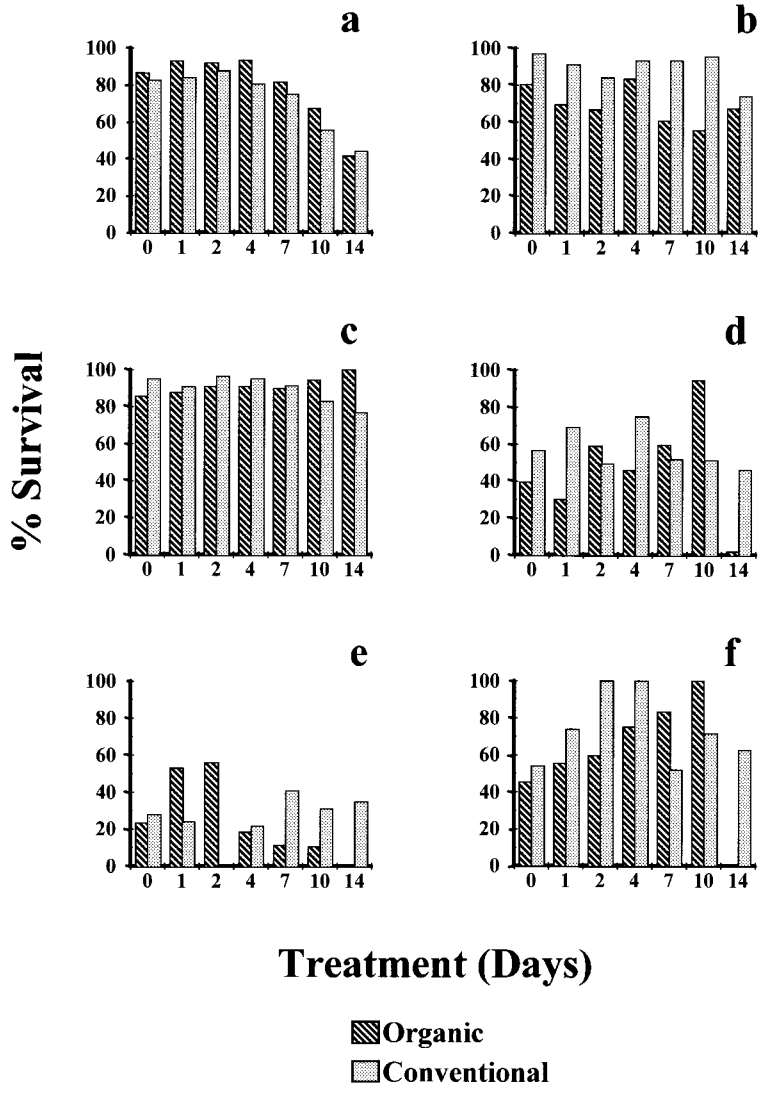

Fig. 3. Survival per life stage of codling moth reared (at 25°C) from organically grown and conventionally produced cherries previously held in cold storage (at 3.3°C) for 0, 1, 2, 4, 7, 10, and 14 d; immature apples were substituted when cherries were consumed by disease. (a) I-instar, (b) II-instar, (c) III-instar, (d) IV-instar, (e) V-instar, (f) pupa.

noticed as missing tended to decrease with increased storage treatments. Only after cold storage for 4 d was there no significant difference between organic and conventionally grown cherries in number of days before larvae were missing ($F = 1.78$; df = 1, 232; $P > 0.05$).

Larval Development. All observed larvae remained as first instars after the cold treatment. The accumulated durations of each subsequent instar were estimated for larvae reared from organic and conventionally grown cherries. The SEM was below 2 d for all categories, indicating little variability in instar development among the cold storage regimes within either organic or conventionally grown cherries (Table 2). Larval development was significantly longer in conventionally produced cherries than in the organically grown fruits. Under normal conditions at 26.7 ± 3°C, the combined larval and pupal period is about 31 d for rearing on artificial diet and immature apples (Hathaway et al. 1971). The delayed larval development time in cherries may be due to nutritional deficiencies or chemicals that may slow the physiological processes and decrease normal body size (House 1965).

The number of codling moths completing the larval stage differed by cold storage treatment (Table 3). Larval survival was lower with organic cherries and with cold storage treatment more than 7 d. Between organic and conventionally grown cherries, only larval survival for the controls (0 d) ($t = 8.40$, df = 6, $P < 0.05$) and for treatment for 2 wk ($t = 24.40$, df = 6, $P < 0.01$) were significantly different. Mature fifth instars can be identified by their uniquely colored head cap-

Table 4. Numbers of adult codling moths completing larval development in cherries and cherries-apples from organically grown and conventionally produced cherries after different durations of cold storage at 3.3°C

Cold storage, d	Organic			Conventional		
	No. adults		% adults	No. adults		% adults
	Cherry alone	Cherry–apple		Cherry alone	Cherry–apple	
0	0	1	0.5	7	6	6.5
1	4	6	5.0	7	10	8.5
2	5	17	11.0	6	10	8.0
4	5	4	4.5	9	7	8.0
7	1	4	2.5	9	5	7.0
10	2	4	3.0	4	6	5.0
14	0	1	0.5	1	6	3.5

Data were pooled from four replicates for each cold storage regime.

sules, and some of those reared from cherries were often about a third of the size of larvae reared from a laboratory colony.

Larval Mortality. Although many larvae disappeared due to the destructive effects of diseased cherries, some cadavers were observed. Mortality within a life stage (missing included) was similar for larvae reared from organic and conventional cherries (Fig. 3). Most of the recorded mortality was in the first instar and the level of survival decreased with increased cold storage starting at 7 d. The high mortality rates for the fourth instar reflect the degree of missing larvae due to diseased fruits. The high mortality levels for fifth instars and pupae may have been due to nutritional deficiencies.

The cold storage treatments used in this study were insufficient to eliminate codling moth in cherries. Furthermore, these treatments were too short to indicate a dose-mortality trend. In previous studies with codling moth eggs, which are less cold tolerant than larvae, complete mortality took between 30 to 42 d (Moffitt and Albano 1972, Moffitt and Burditt 1989). However, cherries are highly perishable and 2 wk is close to their limit for marketable quality.

Adult Emergence. The highest adult emergence (11%) was for organic cherries with 2 d of cold storage (Table 4). The remaining groups had <8% emergence. The majority of the adults came from apples that had replaced deteriorated cherries. There was no significant difference in adult emergence between organic and conventionally grown cherries for each of the cold storage regimes ($t = 0.30$, df = 6, $P > 0.05$). In comparison, 28.6% of larvae emerged as adults in mass rearing (Hathaway et al. 1973) and 48% of larvae emerge as adults from immature apples (Hathaway et al. 1971).

Codling moths have difficulty completing their life cycle on cherries that have been harvested because the fruit cannot remain in good enough condition to allow for complete insect development. Infested fruits are highly susceptible to many types of fungi, causing the fruits to deteriorate quickly, which is detrimental for insect development.

Our research also indicated that the residues on conventionally grown fruits used in this study did not have a measurable detrimental impact on larval survival. Conventionally produced fruits were no less susceptible to insect attack than the organically grown cherries.

The low larval survival indicates that cherries are at least a poor host for codling moth. The relationship between cherries as a resource and larval development demands further examination. Cowley et al. (1992) pointed out that successful pest development in a fruit is a critical criterion in determining host status. Also, it is questionable that codling moth can complete larval development in cherries that are so susceptible to disease and tissue breakdown. Even if fruits were infested with larvae after harvest, they should be easily recognized and eliminated in the packing line. Furthermore, a recent survey in two packing houses of cherries from 63 orchards revealed no codling moth infestation or damage (Rehmke et al. 1998). If it can be established that larval development of codling moth in cherries is suboptimal and does not result in viable adults, a less severe approach can be designed to meet the quarantine requirements for codling moth in sweet cherries (Wearing et al. 2001). Reducing the need of methyl bromide fumigation would benefit both the environment and food quality.

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